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Applicant: MICHEAL L. GRUENBERG

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Art Unit: 1644

Examiner: Schwadron, R

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Michael Lough

MARKED UP CLAIMS (37 C.F.R. § 1.121)

Please amend claim 48 as follows:

48. (Amended) A method of autologous cell therapy comprising:

(a) collecting a tissue or body fluid sample comprising mononuclear cells from a mammal;

(b) activating the cells *ex vivo* in the presence of interferon-gamma, anti-IL-4 antibody or IL-12 to alter their cytokine production profile to produce activated [T] Th1 cells [selected from Th1, Th1-like, Th2 and Th2-like cells];

(c) inducing cell proliferation and cell expansion, in the absence of exogenous interleukin-2, by contacting the activated [T] Th1 cells with [one] two or more mitogenic antibodies to produce a clinically relevant number of [T cells selected from] Th1[, Th1-like, Th2 or Th2-like] cells; and

(d) reinfusing at least 10^{10} cells.

(d) two or more mitogenic antibodies to produce a clinically relevant number of Th1 cells; and

reinfusing at least 10^{10} cells.

REMARKS

A check for the fee for a one month extension of time accompanies this response. Any fee that may be due in connection with this application may be charged to Deposit Account No. 50-1213. If a Petition for extension of time is needed, this paper is to be considered such Petition.

Claims 48 and 126 are presently pending. Claim 48 is amended herein to cancel non-elected subject matter and, and in the interest of advancing prosecution of subject matter in this application to allowance, to conform to a requirement of the Examiner. Claims 51 and 127 are cancelled without prejudice or disclaimer as duplicative of amended claim 48; claim 124 is cancelled without prejudice or disclaimer to avoid possible overlap with claims in a co-pending application.

Basis for amendments of the claims also can be found in the specification at pages 28-34, which describes the agents and signals for Th1 cell differentiation and activation. The Examiner also acknowledges that the specification explicitly discloses the agents listed in claim 48 (see page 3, lines 1-2, of the present Office Action). Therefore, no new matter is added.

A marked up copy of claims showing the amendments herein is appended hereto.

Also provided herewith is an associate Power of Attorney appointing the undersigned to prosecute this application.

REJECTIONS UNDER 35 U.S.C. §112, FIRST PARAGRAPH

Claims 48, 51, 126 and 127 are rejected under 35 U.S.C. §112, first paragraph, as containing subject matter that is allegedly not described in the specification in such as way as to reasonably convey that applicant had possess thereof at the time of filing. Although Applicant respectfully disagrees with the

basis for the rejection, in the interest of advancing subject matter to allowance, the claims as amended recite the agents noted by the Examiner to be described in the specification.

Relevant law

The purpose behind written description requirement is to ensure that the patent applicant had possession of the claimed subject mater at the time of filing of the application In re Wertheim, 541 F.2d 257, 262, 191 USPQ 90, 96 (CCPA 1976). The manner in which the specification meets the requirement is not material; it may be met by either an express or an implicit disclosure.

35 U.S.C. §112 requires a written description of the invention. This requirement is distinct from and not coterminous with the enablement requirement:

> The purpose of the 'written description' requirement is broader than to merely explain how to 'make and use'; the applicant must also convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the 'written description' inquiry, whatever is now claimed." Vas-Cath, Inc. v. Mahurkar, 935 F.2d at 1563-64, 19 USPQ2d at 1117 (emphasis in original).

The issue with respect to 35 U.S.C. §112, first paragraph, adequate written description has been stated as:

> [d]oes the specification convey clearly to those skilled in the art, to whom it is addressed, in any way, the information that appellants invented that specific compound [claimed embodiment] Vas-Cath, Inc. v. Mahurkar, at 1115, quoting In re Ruschig, 390 F.2d 1990, at 995-996, 154 USPQ 118 at 123 (CCPA 1967).

A specification must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention, *i.e.*, whatever is now claimed. Vas-Cath, Inc. v. Mahurkar, 935 F.2d 1555, 1563-64, 19 USPQ.2d 1111, 1117 (Fed. Cir. 1991). A written description requirement issue generally involves the question of whether the subject matter of a claim is supported by or conforms to the disclosure of an application as filed. The test for sufficiency of support in a patent application is

whether the disclosure of the application relied upon "reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter." Ralston Purina Co. v. Far-Mar-Co., Inc., 772 F.2d 1570, 1575, 227 USPQ 177, 179 (Fed. Cir. 1985) (quoting In re Kaslow, 707 F.2d 1366, 1375, 217 USPQ 1089, 1096 (Fed. Cir. 1983)) (see also, MPEP 2163.02).

An objective standard for determining compliance with the written description requirement is "does the description clearly allow persons of skill in the art to recognize that he or she invented what is claimed." In re Gosteli, 872 F.2d 1008, 1012, 10 USPQ.2d 1614, 1618 (Fed. Cir.1989). The Examiner has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. In re Wertheim, 541 F.2d 257, 265, 191 USPQ 90, 98 (CCPA 1976); *See also* Ex parte Sorenson, 3 USPQ.2d 1462, 1463 (Bd. Pat.App. & Inter. 1987). By disclosing in a patent application a device that inherently performs a function or has a property, operates according to a theory or has an advantage, a patent application necessarily discloses that function, theory or advantage, even though it says nothing explicit concerning it. The application may later be amended to recite the function, theory or advantage without introducing prohibited new matter. In re Reynolds, 443 F.2d 384, 170 USPQ 94 (CCPA 1971); and In re Smythe, 480 F. 2d 1376, 178 USPQ 279 (CCPA 1973).

Furthermore, the **subject matter of the claim need not be described literally (i.e., using the same terms or inhaec verba)** in order for the disclosure to satisfy the description requirement. If a claim is amended to include subject matter, limitations, or terminology not present in the application as filed, involving a departure from, addition to, or deletion from the disclosure of the application as filed, the examiner should conclude that the claimed subject matter is not described in that application.

The guideline promulgated by the U.S. PTO embody these rules:

> In rejecting a claim, set forth express findings of fact regarding the above analysis which support the lack of written description conclusion. These findings should:
>
> > (1) identify the claim limitation not described; and
> >
> > (2) provide reasons why a person skilled in the art at the time the application was filed would not have recognized the description of this limitation in view of the disclosure of the application as filed.

in this instance, there is not basis to conclude that a person skilled in the art at the time the application was filed would not have recognized the description of this limitation in view of the disclosure of the application as filed.

Analysis

It is noted, however, that the specification teaches a variety of ways to induce differentiation. For example, at pages 29-30, the specification states:

> Accordingly, in a preferred embodiment, the mononuclear cells collected in the first step of the present process are next activated in the presence of IL-12, interferon-gamma or IL-4 to cause the development of Th1 or Th2 cells, respectively. To enhance the differentiation of regulatory cells, antibodies to IL-12 and/or interferon-gamma can be used to promote Th2 responses, while antibodies to IL-4 can be used to promote the differentiation of Th1 cells. Antibodies or other proteins specific for the IL-12, interferon-gamma or IL-4 receptor on T-cells could also be used to provide a signal in place of the lymphokines. The cells can be activated either non-specifically with chemical agents such as PHA and PMA or with monoclonal antibodies such as anti-CD3 or anti-CD2. Preferably, they are activated specifically with natural or man-made protein antigens added to the medium, processed and presented by APC to T-cells. It may be necessary in some cases to vaccinate the patient prior to blood collection in order to increase the starting number of antigen-specific cells. Another strategy is to oral tolerize patients prior to blood collection. In cases where the cells generated are specific for a known antigen, the antigen may also be used after the cell reinfusion as a booster to increase the desired regulatory cells in vivo. Additional strategies for effecting Th1 cell differentiation is to activate cells in the presence of αB7.2 mAb or TGF-β. Th2 differentiation also can be promoted by activating cells in the presence of one or more of agents, such as, one or more of the following: αB7.1 mAb, low antigen doses and CTLA4/Ig fusion protein (CTLA4 is a ligand for CD28). CD28 is expressed on T-cells and antigen presenting cells.

The specification teaches a variety of ways to achieve differentiation and/or expansion. For example, at page 17, the specification teaches:

> As used herein, activating proteins are molecules that when contacted with a T-cell population cause the cells to proliferate. T-cells generally require two signals to proliferate. Activating proteins thus encompasses the combination of proteins that provide the requisite signals, which include an initial priming signal and a second co-stimulatory signal. The first signal requires a single agent, such as anti-CD3 mAb, anti-CD2 mAb, anti-TCR mAb, PHA, PMA, and other such signals. The second signal requires one or more agents, such as anti-CD28, anti-CD40L, cytokines and other such signals. Thus activating proteins include combinations of molecules including, but [are] not limited to: cell surface protein specific monoclonal antibodies, fusion proteins containing ligands for a cell surface protein, ligands for such cell surface proteins, or any molecule that specifically interacts with a cell surface receptor on a mononuclear cell and indirectly or directly causes that cell to proliferate.
>
> As used herein, a mitogenic monoclonal antibody is an activating protein that is an antibody that when contacted with a cell directly or indirectly provides one of the two requisite signals for T-cell mitogenesis. Generally such antibodies will specifically bind to a cell surface receptor thereby inducing signal transduction that leads to cell proliferation. Suitable mitogenic antibodies may be identified empirically by testing selected antibodies singly or in combination for the ability to increase numbers of a specific effector cell. Suitable mitogenic antibodies or combinations thereof will increase the number of cells in a selected time period, typically 1 to 10 days, by at least about 50%, preferably about 100% and more preferably 150-200% or more, compared to the numbers of cells in the absence of the antibody.

At pages 32-33, the specification teaches:

> In order for T-cells to proliferate, they require two separate signals. The first signal is generally delivered through the CD3/TCR antigen complex on the surface of the cells. The second is generally provided through the IL-2 receptor. In order to bypass the IL-2 signal, combinations of mAb are used. Preferably, the mAb are in the soluble phase or immobilized on plastic or magnetic beads, in order to simplify the cell harvesting procedure.

(i) First signal

To provide the first signal, it is preferable to activate cells with mAb to the CD3/TCR complex, but other suitable signals, such as, but not limited to, antigens, super antigens, polyclonal activators, anti-CD2 and anti-TCR antibodies, may be used. Other suitable agents can be empirically identified. Immobilized or cross-linked anti-CD3 mAb, such as OKT3 or 64.1, can activate T-cells in a polyclonal manner [see, Tax, et al. (1983) Nature 304:445]. Other polyclonal activators, however, such as phorbol myristate acetate can also be used [see, e.g., Hansen, et al. (1980) Immunogenetics 10:247]. Monovalent anti-CD3 mAb in the soluble phase can also be used to activate T-cells [see, Tamura, et al. (1992) J. Immunol. 148:2370]. Stimulation of CD4+ cells with monovalent anti-CD3 mAb in the soluble form is preferable for expansion of Th2 cells, but not Th1 cells [see, deJong, et al. (1992) J. Immunol. 149:2795]. Soluble heteroconjugates of anti-CD3 and anti-T-cell surface antigen mAb can preferentially activate a particular T-cell subset [see, Ledbetter, et al. (1988) Eur. S. Immunol. 18:525]. Anti-CD2 mAb can also activate T-cells [see, Huet, et al. (1986) J. Immunol. 137:1420]. Anti-MHC class II mAb can have a synergistic effect with anti-CD3 in inducing T-cell proliferation [see, Spertini, et al. (1992) J. Immunol. 149:65]. Anti-CD44 mAb can activate T-cells in a fashion similar to anti-CD3 mAb. See, Galandrini, et al. (1993) J. Immunol. 150:4225].

For purposes herein, monoclonal antibodies to anti-CD3 are preferred. Anti-CD3 is used because CD3 is adjacent to the T-cell receptor. Triggering of CD3, such as by monoclonal antibody interaction, causes concomitant T cell activation.

(ii) Second signal

To then cause proliferation of such activated T cells, a second signal is required. A variety of mAb singly or in combination can provide the second signal for T-cell proliferation. Anti-IL-4R mAb (specific for the interleukin-4 receptor molecule) can enhance the proliferation of the Th2 cells [see, Lindquist, et al. (1993) J. Immunol. 150:394]. Immobilized ligands or mAb against CD4, CD8, CD11a (LFA-1), CD49 (VLA), CD45RO, CD44 and CD28 can also be used to enhance T-cell proliferation [see, Manger, et al. (1985) J. Immunol. 135:3669; Hara, et al. (1985) J. Exp. Med. 161:1513; Shimizu, et al. (1990) J. Immunol. 145:59; and Springer, (1990) Nature 346:425]. Cell surface proteins that are ligans to B-cells are preferred targets for Th2 cell proliferation, while macrophage ligands are preferred for Th1 cell proliferation.

Anti-CD28 mAb in combination with anti-CD3 or anti-CD2 induces a long lasting T-cell proliferative response [see, Pierres, et al. (1988) Eur.

J. Immunol. 18:685]. Anti-CD28 mAb in combination with anti-CD5 mAb results in an enhanced proliferative response that can be sustained for weeks [see, Ledbetter, et al. (1985) J. Immunol. 135:2331]. Anti-CD5 mAb alone can also provide a second signal for T-cell proliferation [see, Vandenberghe et al. (1991) Eur. J. Immunol. 21:251]. Other mAb known to support T-cell proliferation include anti-CD45 and CD27 [see, Ledbetter, et al. (1985) J. Immunol. 135:1819 and Van Lier, et al. (1987) J. Immunol. 139:1589].

To determine the combination of mAbs or proteins that optimally induce sustained regulatory cell proliferation, a screening procedure using combinations of these mAbs or proteins is used. The cells are incubated with various combinations of these substances and screened for growth by analysis of ^{3}H-thymidine incorporation or equivalent methods. The group demonstrating the best growth characteristics is selected for use in the medium.

Thus, the specification describes a variety of different agents and combinations thereof for activating and causing proliferation of Th1 cells, and teaches ways for empirically identifying additional agents.

Furthermore, the claims (see original claim 1) as originally filed recite that the method is performed by:

> contacting, in the absence of exogenous interleukin-2, the material with one or more activating proteins specific for cell surface proteins present on cells in the material and in an amount sufficient to induce ex vivo cell expansion, whereby the cells expand to clinically relevant numbers.

Original claim 3 recites:

> The method of claim 1, wherein during the contacting step, the cells in the material are treated under conditions, other than addition of exogenous IL-2, whereby ex vivo differentiation of some or all of the cells into desired effector immune cells is induced.

Original claim 8 recites that the expanded cells are Th1 cells.

Hence, it is clear that applicant appreciated and had possession of the subject matter as claimed at the time of filing of this application and the parent application.

THE REJECTION OF CLAIMS 48, 124, 126 and 127 UNDER 35 U.S.C. § 102(e)

Claims 48, 124, 126 and 127 are rejected under 35 U.S.C. § 102(e) as being anticipated by June *et al.* (U.S. Patent No. 6,352,694) because June *et al.* allegedly discloses that Th1 cells can be produced and expanded using treatment of CD4+ cells with anti-CD3 antibody and anti-CD28 antibody. This rejection is respectfully traversed.

This rejection is respectfully traversed.

Relevant law

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. In re Spada, 15 USPQ2d 1655 (Fed. Cir, 1990), In re Bond, 15 USPQ 1566 (Fed. Cir. 1990), Soundscriber Corp. v. U.S.. 360 F.2d 954, 148 USPQ 298, 301, adopted 149 USPQ 640 (Ct. Cl.) 1966. See, also, Richardson v. Suzuki Motor Co., 868 F.2d 1226, 1236, 9 USPQ2d 1913,1920 (Fed. Cir.), cert. denied, 110 S.Ct. 154 (1989). "[A]ll limitations in the claims must be found in the reference, since the claims measure the invention". In re Lang, 644 F.2d 856, 862, 209 USPQ 288, 293 (CCPA 1981). Moreover it is incumbent on Examiner to identify wherein each and every facet of the claimed invention is disclosed in the reference. Lindemann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co., 730 F.2d 1452, 221 USPQ 481 (Fed. Cir. 1984). Further, the reference must describe the invention as claimed sufficiently to have placed a person of ordinary skill in the art in possession of the invention. An inherent property has to flow naturally from what is taught in a reference In re Oelrich, 666 F.2d 578, 581, 212 USPQ 323, 326 (CCPA 1981).

The claims

Claim 48 includes a step in which cells are activated *ex vivo* in the

presence of interferon-gamma, anti-IL-4 antibody or IL-12 to alter their cytokine production profile to produce activated Th1 cells.

Analysis

June *et al.* does not disclose a differentiation of Th1 cells by treatment with either or both interferon-*γ* and IL-2, or anti-Il-4 antibody or *α*B7.2 mAb or TGF-*β*. Since anticipation requires disclosure of every element as claimed, June *et al.* does not anticipate any of the claims.

THE REJECTION OF CLAIMS 48, 51, 124, 126 AND 127 UNDER 35 U.S.C. §103(A)

Claims 48, 51, 124, 126 and 127 under 35 U.S.C. §103(a) as being unpatentable over June *et al.* (U.S. Patent No. 6,352,694) in view of Sedar *et al.* because June *et al.* teaches that Th1 cells can be produced and expanded using by treatment of CD4+ cells with anti-CD3 antibody and anti-CD28 antibody with out not use exogenous lymphokines, but that June *et al.* fails to teach the use of exogenous lymphokines. Seder *et al.* is alleged to teach that Th1 cells can be produced by treating CD4+ cells with interferon gamma so that it allegedly

> would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have created the claimed invention because June *et al.* teach the claimed method except for the use of interferon gamma treatment (an agent that causes Th1 differentiation), while Seder *et al.* teaches that Th1 (*e.g.*, interferon-gamma producing cells derived from CD4+ T cells) can be produced by treating CD4+ cells with interferon gamma (see page 10190, second column, last paragraph, first sentence).

This rejection is respectfully traversed.

The cited references

June *et al.* is discussed above.

Seder *et al.* merely teaches that activation of cells in the presence of IL-12 or interferon-*γ* leads to the formation of Th1-like cells.

Analysis

As discussed above, the methods taught by June *et al.* differs from the instantly claimed methods because they do not include steps of differentiating cells to produce Th1 cells and then inducing cell proliferation and expanding the cells In the method of June *et al.*, $CD4^+$ cells are contacted with anti-CD3 and anti-CD28. June *et al.* does not indicate any reason, nor does there appear to be any such reason, to differentiate the cells into Th1 cells prior to inducing cell proliferation and expansion. Sedar *et al.* merely teaches that activation of cells in the presence of produces Th1 cells, but does not provide any motivation to modify the method of June *et al.*

The mere fact that prior art may be modified to produce the claimed product does not make the modification obvious unless the prior art suggests the desirability of the modification. In re Fritch 23 USPQ 1780 (CAFC 1992); see, also, In re Papesh, 315 F.2d 381, 137 USPQ 43 (CCPA 1963).

Neither reference suggests the desirability of any modification to the method of June *et al.*; June *et al.* teaches that its methods works very well. Since neither reference suggests the desirability of a modification of the method of June *et al.*, such modification is not obvious.

Babbitt *et al.* in view of Martin *et al.*

Claims 48, 51, 124, 126 and 127 are rejected under 35 U.S.C. §103(a) as being unpatentable over Babbitt *et al.* in view of Martin *et al.* because Babbitt *et al.* allegedly teaches methods of producing Th1 cells and Martin *et al.* teaches administration of 5×10^{10} cells in adoptive T cell therapy. The Examiner concludes that "routine optimization would result in the use of more or fewer cells depending upon the patient" and because Babbitt *et al.* teaches all elements of the claims, except for administration of 10^{10} or 10^{11} cells, which is taught by Martin *et al.*. This rejection is respectfully traversed.

Relevant law

In order to set forth a prima facie case of obviousness under 35 U.S.C. §103, the differences between the teachings in the cited reference

must be evaluated in terms of the whole invention, the prior art must provide a teaching or suggestion to the person of ordinary skill in the art to have made the changes that would produce the claimed product. See, e.g., Lindemnann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co., 730 F.2d 1452, 1462, 221 USPQ 481, 488 (Fed. Cir. 1984). The mere fact that prior art may be modified to produce the claimed product does not make the modification obvious unless the prior art suggests the desirability of the modification. In re Fritch 23 USPQ 1780 (CAFC 1992); see, also, In re Papesh, 315 F.2d 381, 137 USPQ 43 (CCPA 1963).

The Claims

The claims are discussed above.

The cited references

Babbitt *et al.* and differences from the instantly claimed methods

Babbitt teaches a method for production of "immunoreactive cells" by:

(a) contacting a sample of mononuclear cells derived from a patient, e.g., peripheral blood mononuclear cells (PBMC), with OKT3 at or below 37° C to produce an OKT3-derived culture supernatant (T3CS);
(b) removing the T3CS from the sample of patient- derived mononuclear cells;
(c) determining the concentration of OKT3 in the T3CS, and if required, supplementing the T3CS with additional OKT3 to achieve a concentration of at least 0.1 ng/ml;
(d) providing a second sample of mononuclear cells derived from the patient; and,
(e) contacting the second sample of cells with the previously-generated T3CS for a period of time sufficient to yield a population of immunoreactive cells.

Thus, mononuclear cells are obtained and are contacted with T3CS to yield a population of immunoreactive cells. The "immunoreactive cells are described by Babbitt *et al.* as **polyclonal T-cells that exist in a primed state of activation**. Babbitt states that the cells produced by contacting with the T3CS:

> are multifunctional, i.e., possess an enhanced capacity to proliferate and produce cytokines ***upon further stimulation.*** (emphasis added)

These immunoreactive cells are stated to have a:

> low spontaneous level of immune function following processing, but are highly sensitized to respond to *low doses of second signals* up on further culture or *in vivo*.(emphasis added)

At column 4, lines 6-17, Babbitt states:

> Immunoreactive cells have a low spontaneous level of immune function following processing, but are highly sensitized to respond to low doses of second signals upon further culture, or in vivo. The immunoreactive cells of the invention **therefore require further exposure to an immune stimulant,** such as an antigen; target cell, e.g., a tumor cell or virus-infected cell; an inflammatory molecule; an adhesion molecule; an immune cell, e.g., an accessory cell; a cytokine; or any combination thereof, **to achieve full immunologic effector function.** The immunoreactive cells of the invention are multifunctional, polyclonally-activated T cells which have been generated independent of disease-specific antigens utilizing a mixture of nonspecific lymphocyte activators, i.e., autologous cytokines, and a mouse monoclonal antibody, i.e, OKT3, as synergistic stimulants. [emphasis added]

Babbitt continues:

> The ability of EVA cells [the immunoreactive cells] to proliferate and to **produce a variety of cytokines (IL-2, GM-CSF, IFN-gamma, TNF-alpha) in vitro in response to further stimulation by such agents as PMA and IL-2,** as well as to lyse tumor cell targets, is greatly enhanced compared to the PBMC from which they were derived. The lowered activation threshold of the EVA cells exhibited in vitro suggests that once they are reinfused into patients, they are likely to demonstrate enhanced responsiveness to immunological signals, such as weakly immunogenic tumor antigens which normally are non-stimulatory to unprocessed cells. [emphasis added]

Hence the cells produced by exposure to the TC3S medium are immunoreactive cells that require further stimulation. The immunoreactive cells, designated EVA cells, are cells that have low spontaneous levels of immune function following processing and require further signals to function. The Babbitt cells, as stated in Babbitt "display very little spontaneous proliferation or cytokine secretion without PMA stimulation". As stated in Babbitt (see above), the cells must have exogenous IL-2 or other stimulation to **achieve full immunologic effector function.** The cells produced by the Babbitt *et al.* require IL-2 for activation, and, hence are not Th1 cells as defined in the instant application. Furthermore,

T3CS does not contain two or more mitogenic antibodies. It is clear that recitation of two more mitogenic antibodies does not mean two or more molecules of the same antibody, but means different antibodies. .

As discussed above, Babbitt *et al.* is directed to a method for producing immunoreactive cells by contacting a first sample of mononuclear cells with OKT3 at or below 37° C to "produce an OKT-3-derived culture supernatant (T3CS)", removing the T3CS from the mononuclear cells; optionally supplementing the T3CS with additional OKT3 to produce a concentration of at least 0.1 ng/ml of OKT3, and then contacting a second sample of mononuclear cells with the T3CS to produce immunoreactive cells, which Babbitt states require further treatment, such as exposure to Il-2 for activation.

T3CS contains OKT3 antibodies, and may contain IL-1β, IL-6, IL-8, TNFα, TNFβ. IFN-γ, GM-CSF, IL-12, IL-10, Il-4, Il-2 and other cytokines (see, *e.g.*, col. 5, lines 47-63). Thus, patient monocytes are contacted with a composition that contains T3CS, which includes only one, not two or more, activating proteins. Further, the after contacting with the T3CS, the resulting cells are "immunoreactive cells", which as defined by Babbitt *et al.* must be activated by exposure to a second signal.

The cells produced by contacting with T3CS must be exposed to further stimulation to produce cytokines. Th1 cells are defined by their cytokine production, the cells produced by contacting with T3CS are not Th1 cells, until further stimulated. Babbitt *et al.* teaches that such further stimulation requires exposure to IL-2.

At col. 2, lines 57-61, Babbitt *et al.* states:

> By the term "immunoreactive cells" is meant polyclonal T cells that exist in a primed state of activation. Primed cells are multifunctional, i.e., they possess an enhanced capacity to proliferate *and produce cytokines upon further stimulation*. [emphasis added]

Babbitt *et al.* continues at col. 3:

> Immunoreactive cells have a low spontaneous level of immune function following processing, *but are highly sensitized to respond to low doses of second signals upon further culture, or in vivo*. The immunoreactive cells of the invention therefore require further exposure to an immune stimulant, such as an antigen; target cell, e.g., a tumor cell or virus-infected cell; an inflammatory molecule; an adhesion molecule; an immune cell, e.g., an accessory cell; a cytokine; or any combination thereof, to achieve full immunologic effector function. [emphasis added]

The cells, produced by the method, thus require exposure to a second signal, for full immunologic function.

> At co l. 6, lines 19-27, Babbitt *et al.* states:
>
> Following the incubation of cells with T3CS, the cells may be removed from the T3CS and contacted with IL-2, preferably in an amount which is sufficient to bind to at least 25% of the IL-2 receptors on the surface of the immunoreactive cells; more preferably, the amount of IL-2 is sufficient to saturate the IL-2 receptors on the surface of the immunoreactive cells.

Thus, Babbitt *et al.* teaches a method for producing immunoreactive cells that require exposure to IL-2 for full immunologic function. Therefore, the method is different from the instantly claimed methods, which specifically recited that the cells are caused to proliferate and expand in the absence of IL-2.

To produce that T3CS is conditioned medium Babbitt *et al.* only adds a single monoclonal antibody, OKT3. Babbitt *et al.* does not teach or suggest the sue of with two or more proteins that interact with cell surface proteins. Hence Babbitt *et al.* is directed to a method for producing a supernatant that contains a single monoclonal antibody, OKT3, to produce cells that are primed and require subsequent exposure to exogenous cytokines, particularly Il-2. Any teachings disclosure of expansion of the cells contemplates using IL-2 to effect expansion (see column 18, lines 7 -20).

The method of Babbitt *et al.* does not use mitogenic antibodies for inducing proliferation and expansion, but rather uses a supernatant and a single monoclonal antibody to produced primed cells, which then are further treated with Il-2. Furthermore, Babbitt *et al.* does not suggest the step of expanding

the selected cells clinically relevant numbers in the absence of interleukin-2 (IL-2).

Thus, Babbitt teaches a method involving obtaining cells from a patient, treating the cells with a single mitogenic factor OKT3, and optionally adding exogenous cytokines (see, col. 5, lines 2-15), collecting the supernatant from such cells, and contacting the supernatant with additional cells from a patient to produce "immunoreactive cells" that require further stimulation, such as contacting with IL-2 or other exogenous cytokines. Cells produced in the first step by treatment with OKT3 and other cytokines are not the cells for re-infusion into the patient, but are used to produce a supernatant that is used to treat cells, which then are exposed to Il-2 for expansion. Hence, the cells are not expanded in the absence of Il-2 as required by the instant claims.

In contrast the instant methods, include the steps of differentiating leukocytes or mononuclear cells to become Th1 cells, and expanding the resulting Th1 cells in the presence of two or more mitogenic mAbs to produce clinically relevant numbers of cells. Babbitt does not teach or suggest the instantly claimed method. The description in col. 5, referenced by the Examiner refers to addition of cytokines to the T3CS supernatant, which is then used to contact cells to produce immunoreactive cells. Any description of Th1 cells, refers to production of Th1 cells in the first step of the method, which is used to produce the T3CS supernatant. Cells from the patient are then cultured in the supernatant, which contains a variety of cytokines. The resulting cells are the immunoreactive cells, referred to by Babbitt as EVA cells, **not** Th1 cells. As discussed above, the immunoreactive cells, are not Th1 cells; the Th1 cells are the cells that result following treatment of the immunoreactive cells with Il-2.

Finally, Babbitt *et al.* does not teach a method of altering immune balance by producing Th1 cells and infusing at least 10^{10} cells.

Martin *et al.*

Martin *et al.* is directed to a method of determining lymph nodes enriched in tumor reactive cells by administering a radiolabeled locator that specifically binds to a marker associated with neoplastic disease; waiting a predetermined time;, and then testing lymph nodes for accretion of label. Positive lymph nodes are removed and those determined to be tumor-free are cultured to produce tumor-reactive cells. Martin does not teach a method of altering immune balance by producing Th1 cells and infusing at least 10^{10} cells.

Analysis

There would have been no motivation to have combined the teachings of Babbitt with those of Martin *et al.*

Babbitt *et al.* is directed to a method for activating patient-derived mononuclear cells; and Martin *et al.* is directed to a method for identifying lymph notes that contain tumor reactive cells and growing the cells. Hence the two methods are very different, since the starting materials, steps of the methods, and end-products are very different. Therefore, there would have been not motivation to have combined the teachings of Babbitt *et al.* with those of Martin *et al.*

The combination of teachings of Babbitt *et al.* and Martin *et al.* does not result in the instantly claimed methods

Notwithstanding the lack of motivation to have combined the teachings of Babbitt *et al.* with those of Martin *et al.*, the combination thereof does not result in the instantly claimed methods. As discussed above, Babbitt *et al.* is deficient in failing to teach a method in which cells are contacted with two signals in the absence of Il-2. Babbitt *et al.* teaches a method in which a supernatant that contains OKT3 is produced. The supernatant is used to treat additional cells from a patient to produce EVA (*ex vivo* activated) cells that are immunoreactive cells, which as discussed above are **polyclonal T-cells that exist in a primed state of activation** that are:

> are multifunctional, i.e., possess an enhanced capacity to proliferate and produce cytokines ***upon further stimulation.*** (emphasis added)

These immunoreactive cells are stated by Babbitt to have a:

> low spontaneous level of immune function following processing, but are highly sensitized to respond to *low doses of second signals* up on further culture or *in vivo*.(emphasis added)

The cells require Il-2. The method of Babbitt *et al.* does not employ a step of treatment of activated cells with two or more mitogenic antibodies in the absence of exogenous Il-2, and does not teach a method of altering immune balance by producing Th1 cells and infusing at least 10^{10} cells. Martin *et al.* fails to cure any of these deficiencies.

Thus, the combination of teachings of Babbitt and Martin *et al.* does not result in the instantly claimed methods. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

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In view of the above remarks and the amendments and remarks of record, consideration and allowance of the application are respectfully requested.

Respectfully submitted,
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